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# STERILIZATION:- A REVIEW

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## ABSTRACT

Sterilization (or Sterilization ) refers to any process that eliminates, removes, kills or deactivates all forms of life and other biological agent (such as Fungi, bacteria, viruses, space forms, prions, unicellular, etc) present in a specified region, such as a surface a volume of fluid, medication or in a compound such as biological culture media. Dry heat was the first method of sterilization and is a longer process than moist hear sterilization and is a longer process than moist heat sterilization Ethylene oxide (EO, ETO) has treatment is one of the common methods used to sterilize, pasteurize, or disinfect items because of its wide range of materials compatibility. A widely used method for heat sterilization is the autoclave, sometimes called a converter or steam sterilizer. Sterilization can be achieved using electromagnetic radiation such as electron beams, x-rays, gamma rays, or irradiation by subatomic particles. Fluid that would be damaged by heat, irradiation or chemical sterilization, such as drug products can be sterilized by microfiltration using membrane filters - Sterilization is an essential stage in the processing of any product used for parenteral administration, broken skin, mucosal surface or internal organs, sterilization prevent contamination in sterile products. Microorganism can be killed. Eliminated or inhibited by various physical and chemical agent. Several term used to describe the physical processes and chemical agents employed in controlling microorganism sterilization of microbiological materials. Surgical dressing and equipments and other contaminated items is necessary to minimize the health hard associate articles.

**Introduction:**

Sterilization refers to any process that eliminates removes, kills or deactivates all forms of life and other biological agents (Such as Fungi, bacteria, viruses, spore forms, prions, unicellular eukaryotic organisms such as plasmodium etc.) present in a specified region such as a surface a volume of fluid, medication or in a compound such as biological culture media[(2)]. Sterilization can be achieved through various means including : heat, chemicals irradiation, high pressure, and filtration.

Sterilization is an essential stage in the processing of any product used for parenter administration broken skin mucosal surface or internal organs it is process of the sterilization by which an article, surface or medium is freed of all microorganisms either the vegetative or spore state. This are the process of sterilization is microorganisms can be killed eliminated or inhibited by various physical and chemical agent. Sterilization are term used to describe the physical process and chemical agent. Sterilization employed in controlling microorganism. Sterilization are the process of killing or removing bacteria and other form of living microorganisms. Sterilization are the killing bacteria and others Fungi, bacteria, viruses, spores, form, unicellular eukaryotic organism such as plasmodium. Sterilization of microbiological material, surgical dressings and equipments and other contaminated items in necessary to minimize the health hazard associated with these articles.

The main reasons for controlling microorganisms are :

1) To prevent contamination in sterile product.
2) To prevent contamination in aseptic areas which are used or preparation of sterile dosage form and sterility testing.
3) If prevent decomposition and spoilage of food and food products.
4) If prevents transmission of pathogenic microorganism which as responsible for causing disease in plant animals or human beings.

**Sterilization Method :**

The various method used in sterilization can be classified as they are follows.

I. Physical method

a) Dry heat sterilization

E.g. red heat, hot air, incineration, direct flame etc.

b) Moist heat sterilization/ steam sterilization.

e.g. pasteurization, tyndallization, autoclave etc.

c) Radiation / cold sterilization –

i) use of ultra violet rays : UV light.

ii) Ionising radiations : x-rays, gamma rays , beta ray

d) Filtration/ mechanical method :

i) Asbestoses filter.

ii) Sintered glass filter

iii) filter candles

iv) membrane filter.

II) Chemical method :

a) Gaseous method –

e.g.formaldehyde, ethylene oxide etc.

b) By using disinfectant

e.g. cresol, phenol etc.

**I) Physical method :**

This are physical method involves in process by the use of physical means. These process by the use of physical means. These may involves the utilization of heat in the presence of in the absence of moisture of the application of radiations of mechanical filtration.

**a)Dry heat sterilization :-**

Heat is the most reliable and rapid method of sterilization. The killing effect of dry heat is due to protein denaturation. Oxidative damage and the toxic effect of evaluated values of electrolytes.

The time required for sterilization is inversely.Hot air is used to sterilize, glassware, forceps, scalpels, scissors, spatula, swabs, some pharmaceutical substances such as glycerin. Fixed oil liquid paraffin. Propylene glycol, sulphonamides and dusting powder such as kaolin, zinc oxide, starch etc. It is not suitable for surgical dressings, rubbers, plastics, volatile and heat labile substances.

Hot air is a bed conductor of heat and its penetration power is low as compared to moist heat. Hot air ovens are commonly available in pharmaceutical laboratories for drying and sterilization.

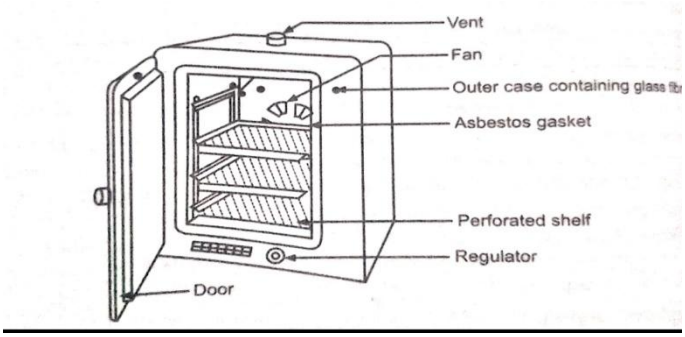


**Fig.1: Hot Air Oven**

**b) Moist heat sterilization:**

Moist heat sterilization describes sterilization techniques that used hot air that is heavily laden with water vapor and where this moisture plays the most important role in sterilization.

Moist hear sterilization is divided into three forms.

i) Temperature below $100^0$c

ii) Temperature at $100^0$c

iii) Temperature above $100^0$c

i) **Temperature below $100^0$c** - Heat labile fluids may be disinfected by heating at temperature below $100^0$c temperature employed is either 63 $^0$c for 30 min (holder method) followed by rapid cooling to $13^0$c or lower This method is known is known as pasteurization products like milk and butter. By this method non-sporing microorganism such as sternum may be disinfected by heating at $56^0$c for one hour. Vaccines prepared from non-sporing bacteria may be inactivated in a water both at $60^0$c for one hour among the moist heat resistant cells are the spone or clostridium balulium which require $120^0$c for four minute.

ii)**Temperature at $100^0$c –** Boiling at $100^0$c for 10 to 30 min wills all vegetable bacteria and some bacterial spores Therefore. It is not recommended for sterilization of instrument for surgical procedures, addition of small quantity of acid alkaloid or washing soda markedly increases the sterilizing power of boiling water.

An atmosphere free steam is used to sterilized culture media which may be decompose if subjected to higher temperature a Koch or Arnold stem sterilizer is usually used this stem sterilizer consists of a vertical metal cylinder with a removable. Conical lid having a small opening for the escaping steam water is added on the bottom and a perfolated shelf above the water level is present single exposure to steam for 90 min ensure complete sterilization but media containing sugar and ghelatine which may get decompose on long heating Hence, such materials may be expose at $100^0$c for 120 min on three successive days.

**iii)Temperature above $100^0$c -** Heat in the form of saturated stem under pressure ismost practical and dependable agent for sterilization. The laboratory apparatus designed to use steam under regulated steam is a more efficient sterilizing agent that hot air because

a) If provides greater lethal action of moist heat.

b) It is quicker in heating up the exposed article and,

c) It can penetrate easily porous material such as cotton wool, stoppers, Paper and cloth wrappers.

The laboratory autoclave or pressure cooker type autoclave consist of a vertical or horizontal cylinder of gun material or stainless steel in a supporting frame or case. The lid is fastended by

serem clamps and rendered air tight by a asbestoses basket. The autoclave has on its life or upper side a discharge tap for air and steam a pressure gauge and a safely, valve that a be set to below of at any desired pressure..

The holding time needed for sterilization are in terms of the temperature and pressure. However the choice of the combination used dependismostlyon the ability of the material to with stands the imposed conditions.

| Temperature ($^0$c) | Steam Pressure | Holding time |
|---|---|---|
| 115-118 | 10 | 30 |
| 121-124 | 15 | 15 |
| 126-129 | 20 | 10 |
| 135-138 | 30 | 3 |

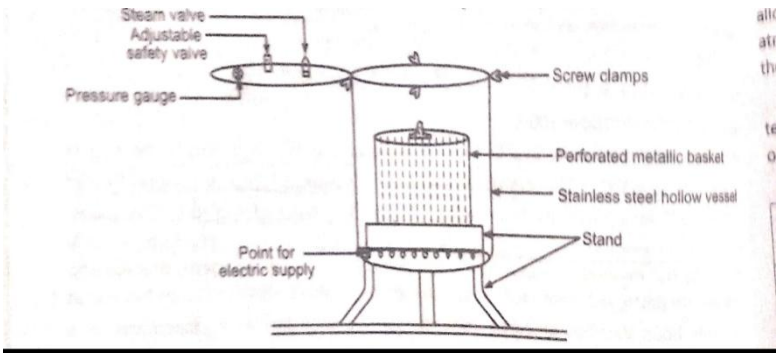


**Fig.2: Vertical Autoclave**

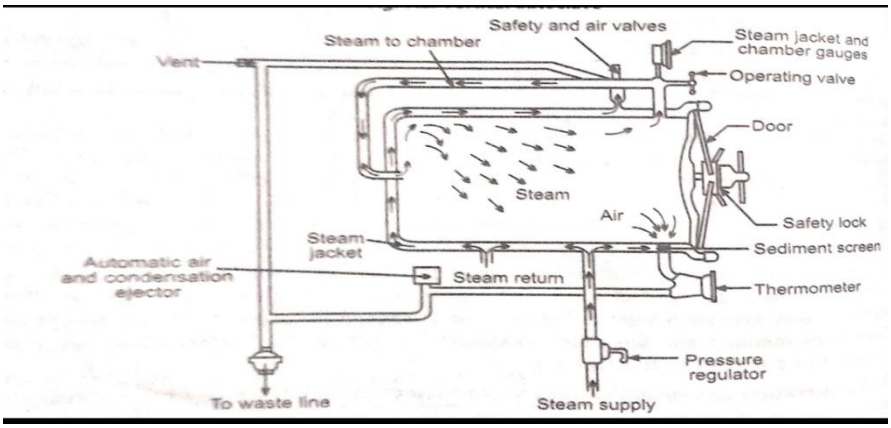


**Fig.3: Horizontal Autoclave**

c) **Radiation –** Energy transmitted through space in a variety of forms is generally called radiation. This method is also called cold sterilization. Sterilization can achieved using electromagnetic radiation, such as electron beams, X-rays, gamma rays, or irradiation by

subatomic particles.Based on their wavelength and penetration power, Radiation can be divided into two categories as non-ionizing radiations and ionizing radiation. Non-ionizing radiations have less energy and do not disturb the atomic Configuration of the target molecule. Ionizing radiations have high energy and ionize the target molecules.

**Non –Ionizing radiations (Ultra violet radiation ) –** Ultraviolet radiation in the region of 2537 $A^0$have been shown to press the greatest activity in destroying microorganism the part of the electromagnetic spectrum between the wavelength of 150-3900$A^0$constitutes UV Radiations. The most common source of artificial UV radiations is UV lamp. These UV lamps are called sterilizing lamps or germicidal lamps

Different biological effects are observed for different types of non-ionizing radition.

Ultraviolet rays are used extensively in hospital operating rooms in asepticFilling room in the pharmaceutical industry, food and dairy industries for treatment of contaminated surface UV rays have also been employed in sterilizing biological fluids such as blood plasma and vaccines.

**Ionizing radiations (Cold Sterilization ) :-** x-rays, gamma rays and cathode rays are highly lethal to DNA and other vital cell constituent They have very high penetration power and considerable energy the factors that effect the lethal activity of ionizing radiations are oxygen protective compound, sanitizing agent, PH of culture , freezing, moisture and recovery conditions.

**a) X-rays** – X-rays have considerable energy and penetration ability that is used to produce lethal effect on microorganism. X-rays can effectively be used for the sterilization of multiple pallet loads of low-densitypackages with very good dose uniformity ration.It is an electricity based and it does not require any chemical or radio-active material.

**b) Gamma- Rays :** Gamma rays are similar –rays but have higher energy and shorter wavelength, Gamma rays are commonly. Gamma rays are commonly obtained using radioactivity isotopes of $60^0$c. Two gamma rays are emitted in a succession as a result of disintegration of almost all of the unstable atoms of the isotopes. The radiant energy particle makes a “Direct Hit” on some essential substances such as DNA within the bacterial cell, Causing ionization which results in the death of cell.

**iii)Cathode rays/ electron beam radiation –**

When a high- voltage potential is established between a cathode and an anode in an evaluated tube the cathode and an anode in an evaluated tube the cathode emits beams of electrons called cathods rays or electrons beams special instrument is the electron accelerator which is extensively used for sterilization of drugs, surgical and other material.

**d)Filtration (Mechanical) method :-**

This is non-thermal method of sterilization used widely in the pharmaceutical industry where heat liable solution are to be sterilized –his is useful for large volume solution eye drops, antibodies solution, sera and carbohydrate solution. This method is also useful for separation of bacterial phages and bacterial toxins from bacteria and for the isolation of microorganism which are scantly in fluids. The process of sterilization by filtration consist of the following main stages for solution.

i ) Passage of the solution through a previously sterilized bacteria proof-filter unit.

ii) Aseptic Transfer of the filtrate to sterile containers which are then sealed aseptically.

iii) Testing of sample for sterility.

Filter efficienkies are affected by their pore size wall thickness, filtration rate, positive or negative pressure and nature of the liquid to be filtered.

**i) Asbestos filter (Setizfilter )**They are disposable single use discs single use discs made up of asbestose. It supported on perforated metal disc within a metal funnel. It is then sited on to a sterile flask through. It is then sited on to a sterile flask through silicone rubber bung the pore size of filter range from 0.01 to 5 micron.

i) **Sintered glass filters (fritted glass filter / mortonfilters )**

Borosilicate glass is fine powered in a ball mill and packed into disc moulds and heated until suitable adhesion takes place between the granules. They have a low adsorptive property and can be cleaned easily. They are brittle and expensive and have a small area of filtration.

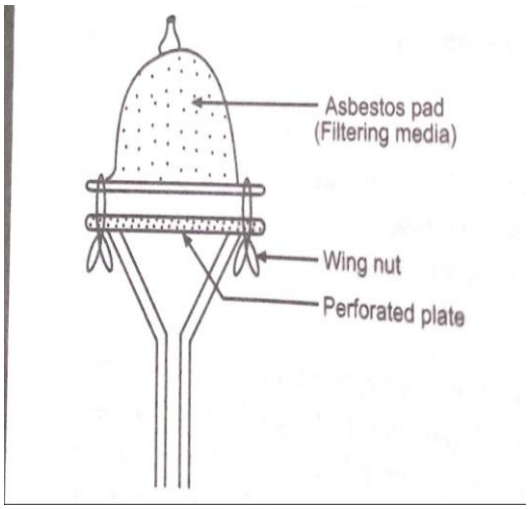


**Fig.4: Seitz Filter**

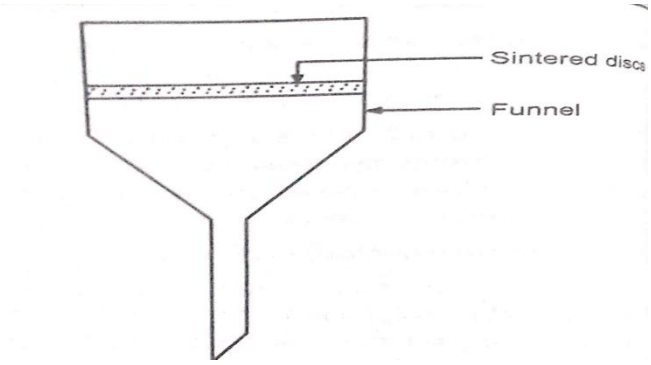


**Fig.5: Sintered Glass Filter**

ii) **Filter candles (Ceramic / bercefieldfilter )**

These are manufactured in different grades of porosity and have been used widely for purification of water for industrial and drinking purposes. They are made of either porous porcelain or kieselguhr. These are depth filter with cellular walls and are available in various sizes.

The filter is fixed to the filter assembly and placed in mentle. The liquid to be filter is poured into the mantle where vacuum forces. It through the filter after fileration, filter candle is removed from the assembly and filtrate& transferred to a sterile container. These filter are inexpensive and available in different sizes. They are easily clogged and blocked and required high pressure for filtration.

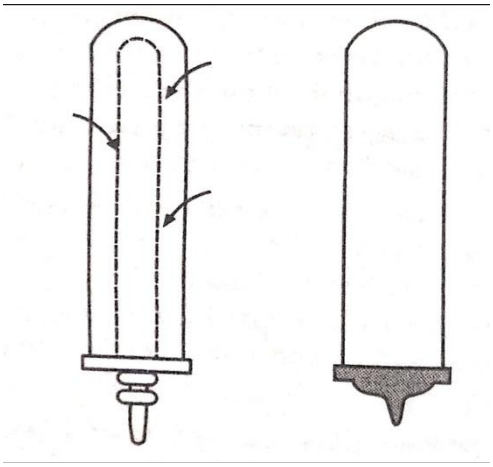

**Fig.6: Filter candles**

iii) **Membrane filter (Millipore / Ultra filter )**

These are made up of various type of cellulose and cellulose esters. They are 150 micrometer thick and contain millions of microscopic pores ranging form 0.01 to 10 micrometer in diameter . The pores sizes most often used for sterilization are 0.45 micrometer $\pm$ 0.02 micrometer or 0.22 micrometer $\pm$0.02 micrometer. Particularly for very small bacterial contaminants. They are sterilized by autoclaving in the holder or packed between thick filter pads to prevent curling. They

are also available at ready sterilized form membrane filters are supported on a rigid base of perforated metal, Plastic or coarse intered glass. The HA grade filters are approximately 65ml/min / Sq. cm with a differential pressure of 10 cm mercury across the membrane.

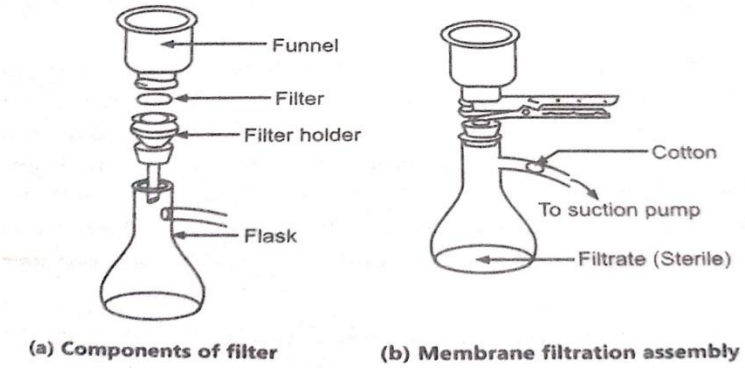


**Fig.7: Member filter**

II) **Chemical methods :**

**a) Gaseous sterilization :-**Gaseous sterilization may be deferred as the destruction of all living microorganism with a chemical in a gaseous or vapor state material substances which are adversely affected by dry and moist hear are than sterilized by this method. All these gases are toxic to human beings above certain concentration and my exhibit other unpleasant or undesirable side effects. Although ethylene oxide is the most widely used gaseous sterilization agent in pharmaceutical and medical field.

The other chemicals used are formaldehyde and B-Propiolactone. In addition to these, various glycols, methyl bromide and alcohol have been used for room sterilization.

i) **Formaldehyde (HCHO) –**This gas is generated by heating a concentrated solution of formaldehyde.

Formalin is vaporized vaporized into formaldehyde gas and then allowed to enter the sterilization chamber which is pre-evacuated and steamed with heated load after the formaldehyde gas pulses are entered, steam and flushed inside.

ii) **B-Propiolactone (BPL) –** This heterotypic ring, compound is a colourless liquid at room temperature with high boiling point.

It is capable of billing all microorganisms and very active against viruses. BPL vaour is approximately 25 times more active as a disinfectant than formaldehyde gas, about 4000 times more active than ethylene oxide and about 50,000 times more active than methyl bromide. It is highly bactericidal and used I concentration of 2 to 5mg / litre. It has a low power of penetration and shows irritation and carcinogenic properties.

iii) **Ethylene oxide :**It is colourless liquid with a boiling point of 10-8$^0$c. it is highly inflammable and may be explosive when mixed with air conc. Greater than 3%.

Its mixtures with carbondioxide or fluorinated hydrocarbons in certain proportions makes ethylene oxide non-inflammable. The carbon dioxide and the freons act as inert dilutents which prevent flammability. Effect of ethylene oxide as a sterilizing agent depends on conc. of gas temperature, moisture, time, conditions and accessibility of the microorganisms.

**Concentration and time relationship commonly used for sterilization.**

| Concentration (mg/lit) | Exposure time (hours) |
|---|---|
| 44 | 24 |
| 88 | 10 |
| 442 | 4 |
| 884 | 2 |

Action of ethylene oxide is due to its power of alkylating the amino carboxyl, hydroxyl and sulphydryl, groups in the enzymes and protein molecule It reacts with DNA and RNA In this reaction the ring in the ethylene oide molecule splits and attaches itself where the hydrogen is present

**b) By using disinfectants or antimicrobial agents :-**Chemical agents most commonly used as disinfectants and antiseptics are phenols, alcohols , halogens, dye, aldehydes etc. These chemical agents and its mode of action, properties

**c) Quantification :**

The aim if sterilization is reduction of initially present microorganism or other potential pathogens, The degree of sterilization is commonly expressed by multiples of the decimal reduction time or D-value, denoting the time needed to reduce the initial number no to one tenth ($10^{-1}$) of its original value [8] . Then the number of microorganisms after sterilization time is given by

$$\frac{N}{N_0} = 10^{\left(\frac{-t}{D}\right)}$$

The D- value is a a function of sterilization conditions and varies with the type of microorganism, temperature, water activity, PH, etc for steam sterilization typically the temperature in degrees celsius is given as an index.

Theoretically, the likehood of the survival of an individual microorganism is never zero. To compensate for this, the overkill method is often used using the over kill method, sterilization is

performed by sterling for longer than is required to kills the bioburden present on or in the item being sterilized. This provides a sterility assurance level equal to the probability of a non-sterile unit. For high –risk applications, such as medical devices and injections a sterility assurance level of at least $10^{-6}$ is required by the united states food ad Drug administration [9]

**Sterilization Criteria :**

**The bioburden :**It is necessary to know the initial number of microorganism present in a given product or association with a given material for selection parameters for any method intended kill microorganism. This initial number is called 'bioburden' or 'bioload'

The time and temperature chosen in such a steam sterilization process is also greatly in excess iof he treatment necessary to kill the small number of heat sensitive contaminatns likely to be present in pharmaceutical likely to be present in pharmaceutical solutions.

**Sensitivity of microorganisms :**Microorganisms show their varying degrees of resistance to heat radiation and chemicals. The vegetative forms of bacteria and fungi are most sensitive. They are about a hundred to thousand times more sensitive to ionization and UV- radiation than the bacterial spores. The thermophilic bacteria smaller viruses and mould spores are filled at temperature between 70to $90^0$c while bacterial spores may be destroy at 90 to $120^0$c temperatures.

**Death rates or survivor curve :** Death in a microbiological population is determined by assessing the reduction in the number of viable microorganism resulting from contact with a given destructive force. This can be represented graphically with a 'survivor curve' drawn from a plot of the logarithm of the fraction of survivors against the exposure time or dose. The death of a population of cells exposed to heat radiation or toxic chemicals is often found to follow first order kinetics.

**Sterility Indicators:-**

It is essential that strict controls are carried out on products to be labeled sterile such controls must then enstate the absence of viable microorganism from these products. There are basically two types of controls.

i) Controls on the process of sterilization i.e. sterilization monitors of sterilization indicators.

ii) Sterility testing of the products.

**1) Physical indicators :**

i) **Moist heat :** A master process record (MPR) is prepared as part of the validation procedure for a particulate autoclave for each specified product and load configuration.

ii) **Dry heat :**Oxidation of cellular constituents is considered the primary lethal process,during dry heat sterilization.

iii) **Radio Sterilization :** A plastic dosimeter gives an accurate measure of the radiation dose absorbed and is considered to the best technique currently available for the radio sterilization process.

iv) **Gaseous methods :**For gaseous sterilization procedures, elevated temperature are monitored for each sterilization cycle made by temperature probles and routine leak. Tests are performed to ensure gas tight seals.

v) **Filtration :** Bubble point pressure test is a technique employed for determining the pore size of filters and may also be used to check the integrity of certain types of filter devices immediately after use. The pressure difference is equivalent to the mean pore size.

**2) Chemical indicators :**Chemical monitoring of a sterilization process is based or the ability of heat, steam, sterilant gases and ionizing radiation to after the chemical or physical characteristics of a variety of chemical substance

i) **Browne's tubes :** The most commonly used chemical indicators for heat process are browne's tube. These are small sealted tubes containing a reaction mixture and an indicator. Exposure to high temperature completes the reaction producing a change in the colour of the indicator.

ii) **Witness tubes :** witness tubes consists & single crystalline substance of known melting point contained in glass tube. A dye may be included to show more clearly that the crystals have metled the crystals have melted. Such a device only indicators that a certain temperature has been reached.

iii) **Heat- Sensitive tape :** Heat sensitive tape is used test this is a test Bowie- Dick, test. This is a test to determine that al air has been reoved from dressings and that subsequent steam penetration has been even and rapid.

iv) **RyceSachet :** The Royce Sachet is a chemical indicator for ethylene oxide sterilization. A polythene sachet containing magnesium chloride, HCL and a bromo phenol blue indicator.

v) **Chemical Dosimeters :**Chemical dosimeters give an accurate measure of the radiation dose absorbed and are considered to be the best technique currently available for controlling radiation sterilization qualitative indicators made of radiosensitive chemicals impregnated in plastic are also available. The indicator changes from yellow to red during irradiation.

3) **Biological indicators :**

Biological indicators constitute of a suitable organism deposited on carrier and are distributed throughout the sterilizing load at the end of the sterilization process the units are recovered and

converted to determine the presence or absence of survivors. The biological indicator measures sterilization process. directly and is able to integrate all sterilization parameters. The selected organism should possess high and reproducible resistance to the sterilizing agent should genetically stable –teadily characterizable and non-pathogenic this viability of the organisms the storage conditions before use and the incubation and culture conditions after sterilization must be standard for the results

**Application :-**

1) Sterilized products which are used in various industries to avoid contamination.
2) It is exposing product to saturated steam at high temperature product to saturated steam at high temperature product are placed in device called the autoclave.
3) It is used various industries include radiation nucleonic gauges.
4) Sterilizing gases are typically used when exposure to heat or radiation or other methods.
5) The appliance uses dry heat for sterilizing of the industrial and laboratory products .
6) Radiation sterilization is generally applied to articles in the dry state.
7) It is used for both the clarification and sterilization of liquids and gases.
8) It is non toxic way of sterilizing things and,
9) It is safe for environment.
10) It is gently and thoroughly penetrates materials
11) Its compatible with metal and sharp objects because of it non corrosive.

**Conclusion :**

When properly used disinfection and sterilization can ensure the sterilization can ensure the safe use of inoscsive and noninvasive medical devices, cleaning should always precede high level disinfection and sterilization current disinfection and sterilization. The method of disinfection and sterilization depends on the intended use of the medical device current disinfection and sterilization guidelines must be strictly followed.

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